REMARKS

Applicants thank Examiner Wegert and Examiner Spector for their time and helpful comments in the interview of July 1, 2004, in which the foregoing claim amendments were discussed and deemed to put the application into condition for allowance. Upon entry of this amendment, *claims 1- 4, 10-11, 13-14, 19, 29, 31, and 35-69 are pending in this application.* The claims have been amended to advance the case to allowance.

Claims 1 and 43 have been amended to claim a preferred embodiment of the invention, wherein the first domain includes an i3-loop peptide or fragment thereof of a GPCR; and wherein the second domain is a cell-penetrating, membrane-tethering hydrophobic moiety. Support for this amendment appears throughout the application, *e.g.,* Table 1 illustrates i3-loop peptides derived from GPCRs such as from PAR1, PAR2, PAR4, SSTR2, CCKA and CCKB. FIG 2A further illustrates i3-loop peptides derived from GPCRs, *e.g.,* from PAR1 and PAR2, including variants having point mutations. Page 11, lines 15-end teaches that activity of the pepducins described in the specification is attributed to "mimicking receptor i3-loop ground-state interactions with the G protein." Support for "cell-penetrating, membrane-tethering hydrophobic moiety" also appears throughout the application. For example, at page 3 line 21 to page 4 line 2, numerous such moieties are disclosed, *i.e.,* "phospholipids, steroids, sphingosines, ceramides, octyl-glycine, 2-cyclohexylalanine, benzolylphenylalanine, propionoyl (C_3); butanoyl (C_4); pentanoyl (C_5); caproyl (C_6); heptanoyl (C_7); capryloyl (C_8); nonanoyl (C_9); capryl (C_{10}); undecanoyl (C_{11}); lauroyl (C_{12}); tridecanoyl (C_{13}); myristoyl (C_{14}); pentadecanoyl (C_{15}); palmitoyl (C_{16}); phtanoyl ($(CH_3)_4$); heptadecanoyl (C_{17}); stearoyl (C_{18}); nonadecanoyl (C_{19}); arachidoyl (C_{20}); heniecosanoyl (C_{21}); behenoyl (C_{22}); trucisanoyl (C_{23}); and lignoceroyl (C_{24})". The aforementioned descriptions are only meant to indicate the support for the claim amendments in the specification and are not limiting on the invention. Dependent claims 4, 10-12, 36 and 39-41 have been amended to be consistent with the new claim language of the independent claim(s). And at the top of page 11, the cell-penetrating, membrane-tethering properties of the claimed hydrophobic moieties are discussed.

Claims 6 and 12 have been cancelled without prejudice or disclaimer. Applicants reserve the right to prosecute cancelled subject matter, as well as the originally presented claims, in continuing applications. New claims 44-68 have been added to individually claim specific embodiments or narrower subgenuses that are already part of the broader, allowable genus claims. Note that support for claims 67 and 68, reciting SEQ ID Nos 28 and 29, may be found in the specification at page 51, paragraph beginning at line 6; and page 53, paragraph beginning at line 2, respectively (see Applicants' amendment of February 6, 2003.) The specification has been amended to include a priority claim to provisional patent application no. 60/198,993.

No new matter has been added by these amendments.

I. Withdrawal of specification and claim objections and rejections

Applicants note with appreciation the withdrawal of the objections to the specification, the objections to claims 3, 4, 8 and 19, and the rejection of claims 1-3, 4, 6, 10-14, 19, 29 and 31 under 35 U.S.C. §112, first paragraph (written description).

II. Rejection of claims 1-4, 6, 10-14, 19, 29, 31 and 35-43 under 35 U.S.C. § 112, first paragraph

The Examiner maintained the rejection of claims 1-4, 6, 10-14, 19, 29, 31 and 35-43 under 35 U.S.C. §112, first paragraph, for improper scope of enablement. Note that claims 6 and 12 have been cancelled herein, so the rejection is moot as it applies to these claims and these claims will not be discussed further. Applicants traverse the rejection as applied to claims 1-4, 10-11, 14, 19, 29, 31 and 35-43 as amended.

The claims have now been amended to require that the first domain of the chimeric polypeptide includes includes an i3-loop peptide or fragment thereof of a GPCR; and a second domain which is a cell-penetrating, membrane-tethering hydrophobic moiety. With respect to the claims as amended herein, they are patentable and overcome the enablement rejection under 35 U.S.C. §112, first paragraph, as set forth below.

Corrections

It will be helpful for a complete understanding of Applicants' comments below to first respectfully correct some discrepancies in the Examiner's comments in the Office Action. The Examiner stated at page 3 of the Office Action that

> "The specification is enabling for a pepducin constructed from the third intracellular loop of the PAR4 receptor, comprising the sequence TLAASG...RRY (SEQ ID NO:9) and attached to the fatty acid palmitate."

However, the Examiner then goes on to say on page 4 that

> "The specification discloses *pepducins* constructed from the third intracellular loop of the PAR1 receptor, comprising the sequence TLAASG...RRY (SEQ ID NO:9) and attached to palmitate." (underlining added.) Applicants point out that "PAR1" should read "PAR4."

The Examiner then goes on to say that

> "Data is presented that demonstrate the pepducins constructed from this sequence of the third intracellular loop, and attached to hydrophobic moieties, interact *intracellularly* with the G-proteins associated with a specific receptor, generally

inhibiting the expected cellular response. For example, pepducins acting at the platelet-aggregation receptors (PAR) inhibit inositol triphosphate production and subsequent platelet aggregation (Specification, Figures 4C and 4D). Additionally, P1pal-13 (the elected invention comprising SEQ ID NO:9) and P1pal-19 were tested for specificity at their cognate receptors versus other G-protein-coupled receptors (Specification, Example 3, paragraphs 121 and 122). (underlining added.)

While the P1pal-13 and P1pal-19 were indeed tested as described by the Examiner, both pepducins do not comprise SEQ ID NO:9. P1pal-13 and P1pal-19 are from *PAR1*; SEQ ID NO:9 is from PAR4.

Scope of Enablement.

Applicants' specification enables the full scope of the claims as presented herein. The present claims recite chimeric polypeptides that include a first domain (which does not comprise a native extracellular portion of a G protein coupled receptor (GPCR)) with an an i3-loop peptide or fragment thereof of a GPCR, and a second domain, attached to the first domain. The first domain binds to its cognate GPCR. The second domain is a cell-penetrating, membrane-tethering hydrophobic moiety.

The first domain

The specification presents several examples of pepducins generated from various and different GPCRs which bind to their cognate GPCR. For example, Table 1 lists - and provides data from the testing of - pepducins generated from six GPCRs: PAR1, PAR2, PAR4, SSTR2, CCKA, and CCKB.[1] Example 8 also describes pepducin modulation of the MC4 obesity receptor.

While the Examiner has appreciated that the specification enables pepducins prepared from PAR4 peptides, Applicants wish to point out that the specification discloses several *other* examples of the synthesis and testing of pepducins generated from PAR1, PAR2, **and** PAR4. *See, e.g.*, Figures 2A, 2B - 2G, 4A - 4D, 6A-6B, 6D, Table 1, page 12, lines 7- page 13, line 10, and *e.g.*, Examples 4 and 6.

The Examples and data presented in Applicants' specification show peptides generated from the many GPCRs noted above, in particular, those made from the third intracellular loop (i3). Without exception and without regard to the GPCR source, all of the pepducins made in the Examples of the specification and in the Declaration were shown to have activity. P1pal-13, P1pal-12, P1pal-7, P1pal-19, P1pal-19Q, P1pal-19E (from PAR1, Example 1) were found to be PAR1 agonists. P2pal-21 and P2pal-21F (from PAR2, Example 4) were found to be PAR2 agonists. P4pal-15 (from PAR4, Example 6), was a full antagonist of PAR4. And

[1] See, *e.g.*, Table 1, also, page 12, lines 7 - page 13, line 10, and, *e.g.*, Example 4.

pepducins based on CCKA, CCKB, SSTR2, MC4 were also found to be agonists. Applicants were able to demonstrate full antagonist activity for PAR1, PAR2 (Fig. 4D), PAR4 (Figs. 4C-D), and other GPCRs such as SSTR2 as summarized in Table 1 of the specification and the Declaration of Dr. Athan Kuliopulos submitted on January 14, 2004.

Thus, Applicants made, tested and disclosed data in their specification on pepducins containing i3-loop peptides derived from *seven* different GPCRs, which, Applicants submit, is sufficient evidence that the breadth of the claims is not greater than what is enabled by the specification.

Applicants further submit that the level of knowledge and skill in the art (at the time the invention was made) is very high, so that one skilled in the art was able to practice the claimed invention. The first domain of the claimed chimeric polypeptides includes an i3-loop peptide or fragment thereof of a GPCR (but not a native extracellular portion of the GPCR), wherein the chimeric polypeptide binds to its cognate GPCR. The highly skilled artisan of this field (GPCR signaling agonism and antagonism) will know how to practice the claimed invention without undue experimentation. First, the structures of GPCRs are well characterized; GPCRs share a highly conserved topological arrangement of a seven transmembrane joined by three intracellular loops, three extracellular loops, and amino- and carboxy-terminal domains.[2] GPCR sequences are, and were at the time of the invention, readily available to researchers. The protein sequences, including those for the third intracellular loop are, and were at the time of the invention, also readily available in public databases and journals. Protein chemistry and synthesis, furthermore, had become a widespread and routine practice in the art at the time of the invention. Thus, obtaining a first domain with which to practice the invention is well within the skilled artisan's ability.

The second domain

The second domain of the claimed chimeric polypeptides is a hydrophobic moiety. The defining characteristics of the hydrophobic moiety are that it be cell penetrating and membrane tethering to mediate delivery of the peptide domain into the cell and anchor it into the cell membrane to facilitate its accumulating at the intraceullar membrane/cytoplasmic interface. For example, the hydrophobic moiety may be a lipid moiety, *e.g.*, a phospholipid, steroid, sphingosine, ceramide, or an amino acid moiety. The claimed hydrophobic moieties can include lipids such as propionoyl (C_3); butanoyl (C_4); pentanoyl (C_5); caproyl (C_6); heptanoyl (C_7); capryloyl (C_8); nonanoyl (C_9); capryl (C_{10}); undecanoyl (C_{11}); lauroyl (C_{12}); tridecanoyl (C_{13}); myristoyl (C_{14}); pentadecanoyl (C_{15}); palmitoyl (C_{16}); phtanoyl ($(CH_3)_4$); heptadecanoyl (C_{17}); stearoyl (C_{18}); nonadecanoyl (C_{19}); arachidoyl (C_{20}); heniecosanoyl (C_{21}); behenoyl (C_{22}); trucisanoyl (C_{23}); and lignoceroyl (C_{24}) (see page 3 line 21 to page 4 line 2). The

[2] Kuliopulos et al., *Life Sciences* **74** (2003) 255-262 at 256.

hydrophobic nature of the second domain allows the chimeric polypeptide to traverse the cell membrane and anchor itself to present the peptide portion in the intracellular space so it may interact with its cognate receptor.

The Examiner stated that a "large quantity of experimentation (was) required to determine how to make all possible chimeric proteins..." Applicants submit that, to the contrary, no such large quantity of experimentation is required. As discussed above, the claims recite that the first domain is an i3-loop peptide or fragment thereof of a GPCR (which can be chosen easily by the skilled artisan as discussed above), and the second domain is a cell penetrating, membrane tethering hydrophobic moiety attached to the first domain. The specification teaches that a wide variety of hydrophobic moieties can be used as the second domain, including those discussed in the previous paragraph. The Examples and data presented in Dr. Athan Kuliopulos' Declaration, of record, clearly demonstrate that various pepducins containing a variety of straight chain lipids and steroids including many of the ones recited above (as well as more complex lipids such as cholate and lithocholate) indeed *work*, just as it is described in Applicants' specification. Applicants submit that this is more than clear enough evidence that the ability to practice the invention as claimed without undue experimentation is well within the purview of one of ordinary skill in the art.

Further Rebuttal of The Examiner's Arguments

Turning to specific other points raised by the Examiner in the Office Action, she stated that

> "(A) sufficient amount of direction or guidance is lacking ... as far as specifying the peptide/fatty acid member that has the binding characteristics and physiological function of P1pal-13 ... The numbers and types of pepducins that can be formed from the claimed G protein-coupled receptor fragments are very large, as well as highly-variable in their physiological effects ...
>
> ...
>
> While the examiner agrees that all or most pepducins produced by the disclosed methods have been shown to be enabled in terms of their interactions with native receptors, each pepducin produced in quite unique in its effects. The instant Specification at Table 1, for example, as well as the Kuliopulos Declaration (15 January 2004, Figures E through I, for example) list results of platelet aggregation or Ca^{2+} fluorescence using many combinations of peptides and lipids comprising the pepducins. All give unique results in terms of affinity, the ability to transduce a response and receptor specificity. This is not surprising given that each lipid differs in

its ability to pass through cell membranes, and each peptide will act differently at each receptor. This is exemplified in Table 1 of the Specification which shows that even pepducins derived from PAR1 (SEQ ID NO's 1-6) each have unique binding and transductional characteristics. Therefore, even though exemplary numbers of pepducins were made and tested, each is unique in its effects and must be claimed with reference to both SEQ ID NO and lipid comprising.

Applicants respectfully point out that the Examiner's reliance on whatever unique effects each pepducin might have is not relevant to determining whether the claims are properly enabled for their scope, and does not require that the claims be limited as urged by the Examiner. What is germane in the end, however, is whether the scope of enablement provided to one skilled in the art by the disclosure is commensurate with the scope of protection sought by the claims. Applicants respectfully submit that the scope of the present claims is proper under 35 U.S.C. §112, first paragraph. One of ordinary skill in the art will not have to engage in undue experimentation to determine possible chimeric polypeptides that have an i3-loop peptide derived from a GPCR. The specification (as well as i3-loop sequences found in the scientific literature) provides a wealth of direction and guidance, as noted above, regarding the sequences or structural requirements necessary for the functional characteristics of the polypeptides embraced by the claims. A more than representative number of working examples are presented to show the use of the claimed polypeptides. The Kuliopulos Declaration provides evidence of both the predictability of the state of the art regarding the claimed constructs, and that undue experimentation *is not* required of the skilled artisan to make and use the claimed invention in its full scope. Therefore, in view of Applicants' amendments and arguments presented in this response, we respectfully submit that the rejection under 35 U.S.C. §112, first paragraph is overcome and request its withdrawal.

REMARKS

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

The Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311 (Reference No. 18475-034).

Respectfully submitted,

Ingrid A. Beattie, Reg. No. 42,306
Nicholas P. Triano, III, Reg. No. 36,397
Attorneys for Applicant(s)
MINTZ, LEVIN, COHN, FERRIS,
GLOVSKY and POPEO, P.C.
One Financial Center
Boston, Massachusetts 02111
Tel: (617) 542-6000
Fax: (617) 542-2241
Customer Number 30623

TRA 1932446v3